REMARKS

In the Office Action, dated February 8, 2006, the Examiner rejected claims 18 and 21 under 35 U.S.C. §102(b) as allegedly being anticipated by "Quantitative Measurement of *Stachybotrys chartarum* conidia Using Real Time Detection of PCR Products with the TaqMan™ Fluorogenic Probe System," R.A. Haugland et al., Molecular and Cellular Probes (1999) 13: 329-340 (hereinafter "HAUGLAND"). The Examiner further rejected claims 19, 20 and 22-27 under 35 U.S.C. §103(a) as allegedly being unpatentable over HAUGLAND in view of "Design Strategies and Performance of Custom DNA Sequencing Primers," Bio Techniques 27:528-536 (September 1999) (hereinafter "BUCK") and GenBank GI: 3420911 (hereinafter "GENBANK"). The Examiner additionally rejected claims 18-27 on the grounds of non-statutory obviousness-type double patenting.

Claims 19, 20, 22 and 25 have amended to improve form. Claims 18-27 remain pending in the application. Applicants request reconsideration of the outstanding rejections of pending claims 18-27 in view of the amendments above and the following remarks.

On page 2, the Office Action rejects claims 18 and 21 under 35 U.S.C. §102(b) as allegedly being anticipated by HAUGLAND. Applicants respectfully traverse.

Independent claim 18 recites a method for identifying and quantifying the presence of the fungus *Stachybotrys chartarum* in a collected sample that includes obtaining a primer set and probe that is specific for the fungal species *Stachybotrys chartarum*, collecting the sample from the environment, extracting the sample's DNA, obtaining DNA standards from a culture of *Stachybotrys chartarum,* determining the concentration of *Stachybotrys chartarum* spores in the DNA standards, amplifying by polymerase chain reaction each of the DNA

standards and the collected sample's DNA using the obtained primer set and probe, and comparing amplification plots obtained by polymerase chain reaction of each of the DNA standards and the collected sample's DNA to obtain an indication of the presence of the fungus *Stachybotrys chartarum* in the collected sample and a concentration of the fungus *Stachybotrys chartarum* in the collected sample.

A proper rejection under 35 U.S.C. §102 requires that a reference teach every aspect of the claimed invention. See M.P.E.P. § 2131. HAUGLAND does not disclose or suggest the combination of features recited in Applicants' claim 18. For example, HAUGLAND does not disclose or suggest, among other features, "obtaining DNA standards from a culture of *Stachybotrys chartarum*", "determining the concentration of *Stachybotrys chartarum* spores in the DNA standards," "amplifying by polymerase chain reaction each of the DNA standards and the collected sample's DNA using the obtained primer set and probe" and "comparing amplification plots obtained by polymerase chain reaction of each of the DNA standards and the collected sample's DNA to obtain an indication of the presence of the fungus *Stachybotrys chartarum* in the collected sample and a concentration of the fungus *Stachybotrys chartarum* in the collected sample," as recited in claim 18.

HAUGLAND discloses a method for performing quantitative PCR to identify and quantify the fungus *Stachybotrys chartarum*. In the method of HAUGLAND, the concentration of spores in the PCR standards is estimated from microscopic counts, and the comparative Ct method (i.e., relative quantitation) is used to quantify the target organism. In contrast to HAUGLAND, Applicants' invention determines the concentration of spores in PCR standards by electronic enumeration, and quantitative PCR (QPCR) (i.e., absolute

quantitation) is used to quantify the target organism. QPCR provides a means of determining the initial concentration of target DNA template in the sample. The theory of quantitation is based on the exponential accumulation of the amplification product during PCR, which is dependent on the concentration of the initial template DNA and the efficiency of the reaction. A quantitation assay can be designed as relative or absolute quantitation. The calculation of the results in the assay (i.e., target concentration) depends on the experimental approach. The method of relative quantitation is used by HAUGLAND to analyze changes in gene amplification in a given sample relative to another reference gene (such as relative increase or decrease -compared to the baseline level- in gene amplification). Relative quantitation includes comparative C_t ($\Delta\Delta C_t$) and relative standard curve methods. The comparative C_t method is essentially an abbreviated version of the relative standard curve method. The relative quantitation method of HAUGLAND involves: performing a run on a real-time PCR instrument, determining the ΔCt value (Target - Endogenous Control), performing the ΔΔCt calculation to determine fold difference in gene expression (ΔCt Target - ΔCt Calibrator), and estimating the target concentration in the sample (see HAUGLAND, p. 330 last sentence of column 1; "...these comparisons can be used to give accurate quantitative estimates of *S. chartarum* conidia in test samples..."). In contrast, the absolute quantitation method of the Applicants involves: determining the absolute quantities of the standards by some independent means (electronic particle enumeration), performing a run on a real-time PCR instrument, setting the measurement threshold and creating a standard curve from the results of amplification of the concentration standards, and determining the absolute target concentration in the unknown samples from the standard curve. The comparative C_t method

utilizes the assumption that the primer efficiencies for amplification of the two different genes are relatively similar. Thus, in assuming this, the standard curve can be omitted from the assay. If the primer efficiencies for both genes are similar, then the comparative C_t method can be used for quantitation. The comparative C_t method offers the advantage of being able to omit the standard curve samples from the experiment, thus increasing the throughput, allowing the simultaneous screening of more samples in one experiment. However, the difference in the primer efficiency between the gene of interest primer set and control primer set should not exceed 5%. Relative quantitation determines the changes in DNA levels of a gene across multiple samples and expresses it relative to the levels of an internal control DNA. This reference gene is often a housekeeping gene and can be co-amplified in the same tube in a multiplex assay or can be amplified in a separate tube (e.g., HAUGLAND used *Geotrichum candidum* as the reference gene). Therefore, relative quantitation does not require standards with known concentrations and the reference can be any gene, as long as its sequence is known. Relative quantitation is based on the comparison of the distinct cycle determined by various methods, e.g. threshold values (C_t) at a constant level of fluorescence.

To summarize, HAUGLAND's quantitation of the target organism is estimated based on the co-amplification of another fungus (i.e., *Geotrichum candidum*) and not on direct comparison to *S. chartarum* standards. HAUGLAND, therefore, does not disclose, or even suggest, the absolute quantitation method of the present application which includes the steps of "obtaining DNA standards from a culture of *Stachybotrys chartarum*", "determining the concentration of *Stachybotrys chartarum* spores in the DNA standards," "amplifying by polymerase chain reaction each of the DNA standards and the collected sample's DNA using

the obtained primer set and probe" and "comparing amplification plots obtained by polymerase chain reaction of each of the DNA standards and the collected sample's DNA to obtain an indication of the presence of the fungus *Stachybotrys chartarum* in the collected sample and a concentration of the fungus *Stachybotrys chartarum* in the collected sample," as recited in claim 18. Since HAUGLAND does not disclose or suggest every feature of claim 18, HAUGLAND cannot anticipate claim 18. Withdrawal of the rejection of claim 18 under 35 U.S.C. §102 is, therefore, respectfully requested.

Claim 21 depends from claim 18. Claim 21, therefore, patentably distinguishes over HAUGLAND for at least the reasons set forth above with respect to claim 18.

In paragraph 8, the Office Action rejects pending claims 19, 20 and 22-27 under 35 U.S.C. §103(a) as allegedly being unpatentable over HAUGLAND in view of BUCK and GENBANK. Applicants respectfully traverse.

In rejecting claims 19, 20 and 22-27, the Office Action asserts (pg. 8) that "[t]he only limitations...not taught by Haugland are the specific primers/probes (SEQ ID NOs 1-5) used for the quantification of *Stachybotrys chartarum*." The Office Action further asserts (pg. 8) that "SEQ ID NOs 1-5 were all well known sequences of the 18S ribosomal RNA gene of Stachybotrys chartarum at the time the invention of the instant application was made as shown by GenBank GI:3420911." The Office Action (pg. 10) additionally alleges that Applicants' claimed primers/probes "simply represent functional homologues of the primers/probes taught by Haugland" and, therefore, that "the claimed primers/probes are *prima facie* obvious over Haugland's primers/probes...." The Office Action further cites to

the disclosure of BUCK as allegedly providing evidence that Applicants' primers are equivalent to the primers disclosed in HAUGLAND and are, therefore, obvious.

A *prima facie* case of obviousness may be made when chemical compounds have *very close structural similarities* and similar utilities. In re Payne, 606 F.2d 303, 313 (CCP 1979). See M.P.E.P. §2144.09. HAUGLAND discloses (pg. 335) a forward primer 5'-TCCCAAACCCTTATGTGAACC-3' and a reverse primer 5'-GTTTGCCACTCAGAGAATACTGAAA-3' for detecting *Stachybotrys chartarum* using polymerase chain reaction. In contrast, the present application claims forward primers (SEQ ID NO: 1) 5'GTTGCTTCGGCGGGAAC3' and (SEQ ID NO: 3) 5'ACCTATCGTTGCTTCGGCG3' and reverse primers (SEQ ID NO: 2) 5'TTTGCGTTTGCCACTCAGAG3' and (SEQ ID NO: 4) 5'GCGTTTGCCACTCAGAGAATACT3'. As can be seen by a comparison of the forward primers of the present application with the forward primer of HAUGLAND, the forward primer of HAUGLAND includes a different sequence of bases then the forward primers of the present application and, therefore, is not structurally similar. As can further be seen by a comparison of the reverse primers of the present application with the reverse primer of HAUGLAND, the reverse primer of HAUGLAND includes a different sequence of bases than the reverse primers of the present application and, therefore, is not structurally similar. Since the primers of the present application are not structurally similar to the primers of HAUGLAND, Applicants submit that the primers disclosed in HAUGLAND do not establish a *prima facie* case of obviousness by themselves.

On page 10, the Office Action cites to In re Deuel and alleges that "since the claimed primers/probes simply *represent functional homologues* of the primers/probes taught by Haugland, the claimed primers/probes are *prima facie* obvious over Haugland's primers/probes..." (emphasis added). From this allegation, it appears that the Office is alleging that a *prima facie* case of obviousness is established because the primers and probe of HAUGLAND perform a similar function (e.g., detection of *Stachybotrys chartarum*) to the primers and probes of the present application, even though the primers of HAUGLAND are entirely different from, and structurally distinct from, the primers of the present application. Applicants respectfully submit that the case law does not support the Office Action's allegation. As noted above, a *prima facie* case of obviousness may be made when chemical compounds have *very close structural similarities*. See M.P.E.P. §2144.09. The case law does not support the proposition alleged by the Office Action that chemical compounds that may perform similar functions are *prima facie* obvious over one another. The case cited by the Office Action, In re Deuel, merely stands for the proposition that the existence of a general method of isolating DNA molecules is irrelevant to the question of whether the specific molecules would have been obvious in the absence of other prior art that suggests the claimed DNAs. See M.P.E.P. 2144.09. In re Deuel has nothing to do with "functional homologs" as alleged by the Office Action and this is apparent from the portion of In re Deuel cited in the Office Action (pg. 10):

> Normally, a prima facie case of obviousness is based upon *structural similarity*, i.e., an established structural relationship between a prior art compound and the claimed compound. *Structural relationships* may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore,

> chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties. (emphasis added)

The above cited section of In re Deuel is concerned with structural similarity between chemical compounds and has nothing to do with "functional homologs" as alleged by the Office Action. Applicants respectfully submit that, since the primers of the present application are not structurally similar to the primers of HAUGLAND, HAUGLAND, by itself, does not establish a *prima facie* case of obviousness. If the Examiner insists on maintaining a rejection based on the allegation that the primers of the present application represent "functional homologs" to the primers of HAUGLAND, Applicants' request that the Examiner provide appropriate citations to authority that demonstrate a legal basis for this allegation.

On page 10, the Office Action further cites to the disclosure of BUCK as allegedly providing evidence that Applicants' primers are equivalent to the primers disclosed in HAUGLAND and are, therefore, obvious. Applicants submit that BUCK does not demonstrate that there is a reasonable expectation of success of achieving the claimed invention and, therefore, that Office Action has failed to establish a *prima facie* case of obviousness.

To make a proper rejection under 35 U.S.C. §103(a), the burden is on the Examiner to establish a *prima facie* case of obviousness. See M.P.E.P. § 2142. As one requirement for establishing a *prima facie* case of obviousness, there must be a reasonable expectation of success of achieving the claimed invention when combining the teachings of the applied references. *In re Vaeck*, 947 F.2d 488, U.S.P.Q.2d 1438 (Fed. Cir. 1991). See M.P.E.P. § 2143. References can, thus, be modified or combined to reject a claim as *prima facie* obvious

as long as there is a reasonable expectation of success. *In re Merck & Co., Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). See M.P.E.P. § 2143.02.

Applicants submit herewith a declaration under 37 C.F.R. § 1.132 in which Dr. Linda Stetzenbach provides her expert opinion, based on an analysis of the HAUGLAND, BUCK and GENBANK references, that the disclosures of these references do not evidence that there was a reasonable expectation of success of deriving the primer and probe set of the present application, that includes primer (SEQ ID NO: 1) 5'GTTGCTTCGGCGGGAAC3', primer (SEQ ID NO: 2) 5'TTTGCGTTTGCCACTCAGAG3', and probe (SEQ ID NO: 5) 6-FAM-5'CTGCGCCCGGATCCAGGC3'-TAMRA, for use in determining a concentration of the fungus *Stachybotrys chartarum* in a sample, where the primers and probe do not cross-react with other fungal species when used in combination in quantitative polymerase chain reaction. Furthermore, Dr. Linda Stetzenbach provides her expert opinion, based on an analysis of the HAUGLAND, BUCK and GENBANK references, that the disclosures of these references do not evidence that there was a reasonable expectation of success of deriving the primer and probe set of the present application, that includes primer (SEQ ID NO: 3) 5'ACCTATCGTTGCTTCGGCG3', primer (SEQ ID NO: 4) 5'GCGTTTGCCACTCAGAGAATACT3' and probe (SEQ ID NO: 5) 6-FAM-5'CTGCGCCCGGATCCAGGC3'-TAM, for use in determining a concentration of the fungus *Stachybotrys chartarum* in a sample, where the primers and probe do not cross-react with other fungal species when used in combination in quantitative polymerase chain reaction.

Dr. Stetzenbach, at the time the invention was made, was the Director of the Microbiology Division of the Harry Reid Center for Environmental Studies at the University of Nevada – Las Vegas and, thus, was the inventors' supervisor. Dr. Stetzenbach was also the Project Director for the U.S. Department of Energy award that funded the study from which the invention resulted. The National Science Foundation additionally provided funds to complete the project. The invention was also part of Applicant Dr. Patricia Cruz-Perez's dissertation, for which Dr. Stetzenbach was Dr. Cruz-Perez's advisor. Dr. Stetzenbach, however, is no longer Director of the Microbiology Division of the Harry Reid Center for Environmental Studies, but is now a Professor in the Environmental and Occupational Health Program of the School of Public Health of the University of Nevada, Las Vegas. Dr. Stetzenbach does not have any financial stake in this invention, or in the issuance of this patent application as a patent. Dr. Stetzenbach's CV is attached as an exhibit to the Rule 132 declaration as evidence of her qualifications and expertise in environmental microbiology and, particularly, in the use of PCR for the detection of environmental microorganisms such as *S.chartarum*.

In view of the facts, opinion and evidence contained in the attached declaration by Dr. Stetzenbach, Applicants respectfully submit that the HAUGLAND, BUCK and GENBANK references do not show that there was a reasonable expectation of success of producing the claimed invention. Since the cited references do not show that there was a reasonable expectation of success, the Office Action has failed to establish a *prima facie* case of obviousness. Withdrawal of the rejection of claims 19, 20 and 22-27 is, therefore, respectfully requested.

On page 11, the Office Action rejects claims 18-27, on the grounds of non-statutory obviousness-type double patenting, as being unpatentable over claims 2 and 17 of U.S. Patent No. 6,733,999 in view of HAUGLAND. Applicants submit herewith a terminal disclaimer. In view of this terminal disclaimer, Applicants respectfully request withdrawal of the rejection of claims 18-27 on the grounds of non-statutory obviousness-type double patenting.

In view of the foregoing amendments and remarks, Applicants respectfully request the Examiner's reconsideration of this application, and the timely allowance of the pending claims. To the extent necessary, a petition for an extension of time under 37 CFR § 1.136 is hereby made.

Respectfully submitted,

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